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S2.P21

The role of the mitochondria-shaping protein Opa1 in the BCR vs. OxPhos subset of diffuse large B cell lymphoma

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Diffuse large B cell lymphomas (DLBCLs) are a genetically heterogeneous group of tumors that can be further divided in several subsets, identified by their distinct molecular signatures. Genome wide arrays and multiple clustering algorithms defined a B cell receptor/proliferation cluster (BCR–DLBCL), which displays the upregulation of genes encoding BCR signaling components, and an OxPhos cluster (OxPhos–DLBCL) which is enriched in genes involved in mitochondrial oxidative phosphorylation. The OxPhos subset lacks an intact BCR signaling network, suggesting dependence on alternative survival mechanisms, which are not yet defined. A proteomic analysis identified increased levels of the mitochondria shaping protein Optic atrophy 1 (Opa1), that regulates mitochondrial fusion and cristae biogenesis, in the OxPhos subset. Here we present evidence that mitochondrial morphology, metabolism, and ultrastructure are different between the BCR and the OxPhos subsets that display different levels of Opa1. Our data indicate a role for Opa1 in DLBCL features.

doi:[10.1016/j.bbabbio.2014.05.309](https://doi.org/10.1016/j.bbabbio.2014.05.309)

S2.P22

Submitochondrial protein distributions at the nanoscale

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Mitochondria exhibit a complex architecture which serves to separate different biochemical pathways. Two membranes divide the interior of the organelle into several reaction rooms. The smooth mitochondrial outer membrane surrounds the entire organelle. At contact sites, this membrane is connected to the highly folded inner membrane. Relatively little is known on the distribution of proteins within these membranes. Arguably, the most suitable approach to quantitatively study the distribution of protein complexes in intact cells is far-field fluorescence microscopy. Because mitochondria exhibit a diameter of around 300 nm, which is in the range of the diffraction limited resolution of conventional light microscopes (~200 nm in the focal plane), a detailed analysis of the distribution of proteins within mitochondria is challenging or even impossible with conventional light microscopy. In this study, we

employed diffraction-unlimited stimulated emission depletion (STED) microscopy together with automated algorithms for image analysis to investigate sub-mitochondrial distributions of various proteins. Amongst others, we found components of the translocase of the mitochondrial outer membrane (TOM complex) to be mainly concentrated in individual clusters whose nanoscale distribution is finely adjusted to the cellular growth conditions. The distributions of these clusters follow an inner-cellular gradient from the perinuclear to the peripheral mitochondria. The nanoscale distribution of many mitochondrial proteins is finely adjusted to the cellular conditions, resulting in distribution gradients both within single cells and between adjacent cells.

doi:[10.1016/j.bbabbio.2014.05.310](https://doi.org/10.1016/j.bbabbio.2014.05.310)

S2.P23

The composition of pea mitochondrial supercomplexes under cold conditions

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Mitochondrial complexes of the respiratory chain in a variety of organisms are arranged in respiratory supercomplexes, respirasomes, and even larger linear structures, so-called megacomplexes [1, 2]. At the same time these supramolecular assemblies and free populations of the complexes I and IV coexist. According to the latest data, the composition and activity of supercomplexes can be reversibly modified upon specific environmental conditions [2]. Cold is one of the major nature factors that impose limitation on plant development. To study the cold-related variation in the composition and activity of specific complexes within the various supercomplexes we analyzed composition and abundance of OXPHOS supercomplexes during cold hardening, mild cold stress and short-term negative temperature treatment in pea (*Pisum sativum*) seedling mitochondria. The investigation of respiratory complexes in pea seedlings mitochondria based on mild solubilization with digitonin and native separation by BN-PAGE revealed that electron transfer chain of mitochondria isolated from pea seedlings mainly consist of five supercomplexes: (1) major supercomplex I + III₂; (2) three supramolecular structures comprising I, III and IV complexes in different ratios, also called respirasomes; (3) and 1 distinct megacomplex, which includes II, III and IV complexes. We determined changes in the activity of complex I and complex IV within some supercomplexes and dissociation of complex I from supercomplexes under low temperature stress conditions. Cold hardening didn't lead to such I complex disassembly. This response of the supercomplex composition could probably present a regulatory mechanism involved in optimization of respiratory activity of pea mitochondria depending on environmental conditions.

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doi:[10.1016/j.bbabbio.2014.05.311](https://doi.org/10.1016/j.bbabbio.2014.05.311)